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(54) **In vitro generation of human dendritic cells and uses thereof.**

(57) A method is provided for generating human dendritic cells in vitro by treating $CD34^+$ cells with tumor necrosis factor-α and either interleukin-3 or GM-CSF. The invention also includes cellular compositions of dendritic cells produced by this method. Dendritic cells of the invention can be used widely as components in many diagnostic and therapeutic systems, including improved mixed lymphocyte reactions for assaying tissue rejection, adoptive immunotherapy of cancer, adoptive immunotherapy of HIV infections, and SCID-hu mice for human antibody production.

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The invention relates generally to an in vitro method of generating human dendritic cells, and more specifically, therapeutic and diagnostic uses of the generated cells.

Dendritic cells are a system of antigen presenting cells that function to initiate several immune responses such as the sensitization of MHC-restricted T cells, the rejection of organ transplants, and the formation of T cell-dependent antibodies. Dendritic cells are found in many nonlymphoid tissues but can migrate via the afferent lymph or the blood stream of the T cell-dependent areas of lymphoid organs. They are found in the skin, where they are named Langerhans cells and are also present in the mucosa. They represent the sentinels of the immune system within the peripheral tissues where they can acquire antigens. As these cells express CD4 and can be infected in vitro by HIV, they are likely to represent a port of entry of HIV virus in vivo, e.g. Knight et al, pp.145 in Racz, et al, editors, Accessory Cells in HIV and Other Retroviral Infections (Karger, Basel, 1991); Ramsauer et al, pp. 155 in Racz, et al, editors (cited above). The isolation of human dendritic cells from peripheral blood has only been recently achieved and only small numbers of cells can be generated, e.g. Freudenthal et al, Proc. Natl. Acad. Sci., Vol. 87, pp. 7698 (1990). The in vitro generation of large numbers of human dendritic cells would represent an important advantage for priming in vitro human naive CD4 and CD8 T cells, for screening agents which may interfere with HIV infection, for generating primary and secondary in vivo response of human B cells in SCID-hu mice reconstituted with human T and B cells, and for constructing a more sensitive mixed lymphocyte reaction assay.

A major impediment to allogenic tissue and organ transplantation is graft rejection by the transplant recipient. The cell-mediated immune reaction of the recipient, or host, to the donor tissue plays an important role in the rejection process. The cell-mediated immune response has two important phases: (i) recognition, when host cells recognize the donor cell as foreign in the context of the major histocompatibility complex (MHC), and (ii) destruction, when the host cells respond by attacking the foreign cells. As part of the attacking process, a number of responder cells undergo proliferation and acquire cytotoxicity--that is, the ability to kill donor cell displaying the appropriate antigens. Thus, cell-mediated immunity can be described in terms of two measurable functions: proliferation and cytotoxic activity, Dubey et al, chapter 131 in Rose et al, Editors, Manual of Clinical Laboratory Immunology, 3rd edition (American Society of Microbiology, Washington, D.C., 1986).

Development of cell culture techniques has led to the establishment of in vitro methods which mimic the in vivo immunization process, thus providing measures for the assessment of cell-mediated immunity in vitro. Of particular utility in regard to transplantation is the mixed lymphocyte response (MLR), or mixed lymphocyte culture. The MLR is a relatively simple assay, yet it exists in many variants. Typically, the assay consists of mixing in a suitable culture system responder lymphocytes with stimulator lymphocytes whose proliferation and/or transcription machinery has been disabled, e.g. by irradiation. After culturing the cells for several days, a number of different measurements can be made to quantify the degree of reactivity of the responder cells to the stimulator cells, e.g. uptake of tritiated thymidine, number of blast cells, number of dividing cells, cytokine production, and the like. Other variables in the assay include the source of the responder and stimulator cells, e.g. peripheral blood, spleen, lymph nodes, etc.; whether the responder cells are syngenic, allogenic, or xenogenic with respect to the stimulator cells; the method of disabling the stimulator cells, e.g. irradiation, treatment with a DNA synthesis inhibitor, e.g. mitomycin C, or the like.

A drawback of the MLR as a routine assay for cell-mediated immune reactivity is sensitivity. Frequently, it is difficult to obtain a strong effect in the MLR, whatever the particular read-out employed. It is believed that antigen presenting cells in the stimulator population are responsible for stimulating the responder cells; however, in most tissue sources such cells few in number and/or are of a type that stimulates inefficiently. The sensitivity, and hence the utility, of the MLR assay could be greatly enhanced by the availability of more potent stimulator cell populations. Dendritic cells could serve this function, as they are well known as potent antigen presenting cells, e.g. Steinman, Ann. Rev. Immunol., Vol. 9, pgs. 271-296 (1991). Unfortunately, it is presently very difficult to obtain them in quantities sufficient for routine MLRs, e.g. Steinman, et al, chapter 49 in Herzenberg et al, Editors, Cellular Immunology, Vol. 2 (Blackwell Scientific Publications, Oxford, 1986).

Summary of the Invention

The invention is directed to a method for in vitro generation of human dentritic cells. The invention also includes isolated populations of human dendritic cells produced the the method of the invention and applications of the isolated cells, including an improved MLR assay that employs a pure population of dendritic cells as stimulator cells. The method of the invention includes the steps of culturing $CD34^+$

hematopoietic progenitor cells in the presence of tumor necrosis factor-α (TNF-α) and either granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin-3 (IL-3) to form $CD1a^+$ dendritic cells of the invention.

Description of the Figures

Figure 1 illustrates flow cytometry data showing the partial co-expression of CD14 by $CD1a^+$ cells. After 12 days of culture in GM-CSF and TNF-α, cells generated from $CD34^+$ cord blood hematopoietic precursor cells were processed for double color fluorescence measurement. Briefly, cells were sequentially incubated with unconjugated monoclonal antibodies, phycoerythrin (PE)-conjugated anti-mouse immunoglobulin, normal mouse serum, and OKT6 (anti-CD1a from Ortho) or Leu-M3 (anti-CD14 from Becton-Dickinson) monoclonal antibodies directly labelled with fluorescein isothiocyanate (FITC). Figure 1A shows two-color fluorescence intensity from IgG_1-FITC isotype control versus IgG_{2a}-PE isotype control. Figure 1B shows two-color fluorescence intensity from OKT6-FITC (proportional CD1a expression) versus Leu-M3-PE (proportional to CD14 expression).

Figure 2 illustrates data concerning the growth kinetics of $CD1a^+$ cells under various medium conditions: In all experiments 10^5 $CD34^+$ cells were expanded under culture conditions described below (i) in the presence of IL-3 (solid triangles), (ii) IL-3 and TNF-α (open triangles), (iii) GM-CSF (solid squares), and (iv) GM-CSF and TNF-α (open squares). Solid lines indicate total cell numbers in the three experiments, and dashed lines indicated expression of CD1a antigen. Figures 2A and 2B represent data from two separate sets of experiments.

Figure 3 illustrates data of $CD4^+$ cell proliferation after stimulation by dendritic cells of the invention. $CD34^+$ cells were cultured for 12 hours in accordance with the invention, after which they were irradiated with 4000 Rads to form stimulator cells for the experiment. From 10 to 2.4×10^4 stimulator cells were seeded for 2.5×10^4 resting $CD4^+$ T cells or other responder cells in round-bottomed microtest tissue culture plates in medium supplemented with 10% human AB^+ serum, as described below. In Figure 3A $CD34^+$ cells were cultured in the presence of either IL-3 (solid triangles), IL-3 and TNF-a (open triangles), GM-CSF (solid squares), or GM-CSF and TNF-α (open squares) and the responder cells were allogenic $CD4^+$ T cells. In Figure 3B, the $CD34^+$ cells were cultured in the presence of GM-CSF and TNF-α for all experiments and the responder cells were adult peripheral blood (open squares), cord blood, (open and solid triangles), and syngenic cord blood $CD4^+$ T cells (solid squares).

Figure 4 illustrates data showing the effect of $CD1a^+$ cells in stimulation of allogenic $CD4^+$ T cell proliferation. After 12 days of culture in the presence of GM-CSF and TNF-α, $CD1a^+$ cells were depleted from a sample of the culture as described below. After irradiation, the following were used as stimulator cells: total population (solid squares), population from which $CD1a^+$ cells had been removed by depletion (open squares), and control population from which depletion was carried out with an anti-IgG_1 isotype antibody (open triangles).

Detailed Description of the Invention

An important aspect of the invention is the generation of dendritic cells from $CD34^+$ hematopoietic cells. $CD34^+$ hematopoietic progenitor cell can be obtained from a variety of tissue sources, e.g. bone marrow, but are preferably obtained from umbilical cord blood samples as follows: Light density mononuclear cells from the samples are isolated by Ficoll-Hypaque gradient separation (d = 1.077 g/mL) and are depleted of adherent cells, e.g. by overnight incubation at 37 • C in RPMI 1640 medium supplemented with 1% w/v tissue-culture grade bovine serum albumin. Preferably, cells bearing CD34 antigen are isolated from nonadherent mononuclear fractions through positive selection by indirect immune panning using anti-CD34 monoclonal antibody, e.g. Imu-133.3 available from Immunotech (Marseille, France), anti-My 10 available from Becton Dickinson (Mountain View, California), or the like. Panning flasks are prepared as follows: sheep Fab antimouse IgG at a concentration of 25 µg/mL in Tris buffer (0.05 mol/L, pH 9.4) is distributed (10mL) in 75-cm^2 tissue culture flasks for overnight coating at 4 • C. Separately, the light-density mononuclear cells (depleted of adherent cells as discussed above) are incubated one hour at 4 • C with 5 µg/mL anti-CD34 antibody at 10^7 cells/mL in RPMI 1640 supplemented with 2% heat-inactivated pooled human AB serum (HABS). Afterwards, cells are washed in cold medium containing 2% HABS, and 10mL containing about 5×10^7 cells are distributed to the flasks previously coated with sheep antimouse IgG, as described above. Following a two hour incubation at 4 • C, nonadherent cells in suspension, (i.e. the CD34-depleted fraction) are harvested by gentle pipetting and rinsing several times with medium. The adherent "panned" cells (i.e. the CD34-rich fraction) are then recovered by vigorous pipetting.

The dendritic cells are obtained from the $CD34^+$ cells by culturing the latter in medium containing TNF-α and either GM-CSF or IL-3. Preferably, the dendritic cells are obtained from the $CD34^+$ cells by culturing the latter in medium containing TNF-α and GM-CSF. TNF-α, GM-CSF, and IL-3 suitable for use in the invention are commercially available, e.g. from Genzyme Corp. (Cambridge, MA), or can be produced by recombinant expression systems, e.g. as taught by Clark et al, U.S. patent 4,959,455 (IL-3); Clark et al, PCT application N° EP85/00326 (publ. N° W086/00639) (GM-CSF); and Mark et al, U.S. patent 4,677,063 (TNF-α). Preferably, IL-3 and GM-CSF are used at saturating concentration. That is, they are used at a concentration in which all the IL-3 and GM-CSF receptors on the $CD34^+$ cells are occupied by the biologically active IL-3 and GM-CSF molecules. Of course, the actual concentration may depend on the quality of IL-3 and GM-CSF used. Preferably, human IL-3 having a specific activity of at least 5×10^6 U/mg is employed, wherein a unit of activity corresponds to the half-maximum proliferative activity as determined by ^3H-thymidine uptake by human bone marrow cells in liquid cultures. In the culture systems described below, saturating concentration was 10ng/mL (or 50 U/mL). Preferably, human GM-CSF having a specific activity of at least 2×10^6 U/mg is employed, wherein a unit of activity is defined the same as that for IL-3. In the culture systems described below, saturating concentration was 100 ng/mL (or 200 U/mL). Preferably, TNF-α is used at a concentration in the range of 2 to 3 ng/mL or 40-60 U/mL. Most preferably, TNF-α is used at a concentration of 2.5 ng/mL, or 50 U/mL. Units of TNF-α are defined by Carswell et al, Proc. Natl. Acad. Sci., Vol. 72, pg. 3666 (1975); and Aggarwal et al, J. Biol. Chem., Vol. 260, pg. 2345 (1985). The cells can be co-cultured in standard tissue culture medium with standard additives, such as RPMI 1640 supplemented with 10% (v/v) heat inactivated fetal bovine serum, 10mM Hepes, 2mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, penicillin (100 U/mL) and streptomycin (100 mg/μL). Preferably, the $CD34^+$ cells are cultured in the presence of the cytokines for between 8 to 12 days.

Dendritic cells that form in the culture are isolated by panning as described above, with the exception that anti-CD1a and/or anti-CD14 antibodies are employed (both antibodies being commercially available, e.g. Becton-Dickinson).

The MLR method of the invention comprises the following steps: (1) providing a sample of responder cells; (2) providing a sample of inactivated stimulator cells such that the stimulator cells are allogenic with respect to the responder cells and such that the stimulator cells consist of dendritic cells produced by the process comprising the steps of (a) treating $CD34^+$ hematopoietic cells with tumor necrosis factor-α and a cytokine selected from the group consisting of granulocyte-macrophage colony stimulating factor and interleukin-3, and (b) isolating treated $CD34^+$ hematopoietic cells that express the CD1a antigen; (3) co-culturing the responder cells and the inactivated stimulator cells; and (4) measuring a response of the responder cells.

Preferably, the sample of responder cells consist of $CD4^+$ T cells from the peripheral blood of a patient who is to be the recipient of a transplant. Obtaining T cell populations employs techniques well known in the art which are fully described in DiSabato et al, eds., Meth. in Enzymol., Vol. 108 (1984). For example, the $CD4^+$ T cells can be isolated as follows: first mononuclear cells are isolated from the peripheral blood and depleted of adherent cells; $CD4^+$ T cells are then purified by depleting other cell types, e.g. by immunomagnetic depletion (e.g. with Dynabeads, Dynal, Oslo, Norway), or the like, using a cocktail of commercially available monoclonal antibodies, e.g. anti-CD14, anti-CD16, anti-CD20, anti-CD8, anti-CD40 (available from Becton-Dickinson and/or Ortho Diagnostic Systems, New Jersey). $CD4^+$ populations having greater than 95% purity are typically achieved after two rounds of immunomagnetic depletion.

Stimulator cells of the invention are dendritic cells derived from $CD34^+$ hematopoietic progenitors cells obtained from a different individual than that from whom the responder cells are taken; that is, the stimulator cells are allogenic with respect to the responder cells. These cells are obtained as described above.

The stimulator cells of the invention are inactivated so that they carry out their stimulatory function, but are inhibited from any other function that could obscure the response measured from the responder cells. Thus, the nature of the inactivation depends somewhat on the "read-out" of the assay. Preferably, the read-out, or response measured in the responder cells, is cellular proliferation. Other read-outs could also include such phenomena as cytokine production, cytolytic ability, and the like. Preferably, the stimulator cells are treated so that they are incapable of replication, but that their antigen processing machinery remains functional. This is conveniently accomplished by irradiating the cells, e.g. with about 1500-3000 R (gamma or X-radiation) before mixing with the responder cells.

Preferably, proliferation of the responder cells is determined by the uptake of tritiated thymidine using standard protocols. For example, from 10 to 2.5×10^4 stimulator cells are added to 2.4×10^4 allogenic $CD4^+$ T cells in 96-well round bottom tissue culture plates and are incubated for 4 days in the medium described above. After incubation, the cells are pulsed with 1μCi of tritiated thymidine for 6 hours, and then they are harvested and measured for tritiated thymidine uptake, e.g. by scintillation counting.

EXPERIMENTAL

Example 1

Generation of Human Dendritic Cells

As shown in Fig. 1, culturing $CD34^+$ cells for 12 days in the presence of GM-CSF and TNF- allows the appearance of $CD14^+$ monocytic cells co-expressing the CD1a antigen. In addition, a population of $CD14^-CD1a^+$ was consistently observed which represented 30-90% of the total CD1a population (range from 5 experiments). Within the monocytic lineage, expression of CD1a antigen is restricted to Langerhands cells. As shown in Fig. 2, $CD1a^+$ cells were not detected at the onset of the culture and the CD1a antigen could first be observed after 4 days of culture in the presence of TNF- and expression increased until day 20. Whereas less than 5% of $CD1a^+$ cells were observed in the presence of IL-3,5-15% $CD1a^+$ cells were detected in GM-CSF. A high proportion of $CD1a^+$ cells was detected in IL-3 and TNF-α (10-25%) and mainly in GM-CSF and TNF-α (20-60%) (range of 5 experiments after 12 days of culture). In terms of growth efficiency, IL-3 and TNF-α is 2-3 times more potent than IL-3 alone, and GM-CSF and TNF-α is 3-4 times more potent than GM-CSF alone (Fig. 2). Thus, starting from 10^5 $CD34^+$ cells, after 12 days in culture, GM-CSF allowed the generation of 1-3 x 10^6 $CD1a^+$ cells, while in IL-3 plus TNF-α and GM-CSF plus TNF-α cultures of 2-3 x 10^6 cells were recovered. As GM-CSF plus TNF-α appeared to be the most potent combination of factors for generating $CD1a^+$ cells, all the cultures for the characterization of those cells contained GM-CSF plus TNF-α.

The morphology of the $CD1a^+$ cells generated by the method of the invention was studied using both light and electron microscopy. Adherent cells observed in GM-CSF alone display the classical aspect of regularly shaped macrophages, whereas those obtained in the presence of GM-CSF plus TNF-α have a typical aspect of dendritic cells with highly ramified dendrites, lobulated nucleus, and a villous surface with dendritic projections. Some of the $CD1a^+$ cells (about 1 in 5) possess organelles with double membrane joining, recalling the structure of Birbeck granules.

The phenotype of $CD1a^+$ cells generated after 12 days of culture in the presence of GM-CSF and TNF-a was determined by double color fluorescence analysis. Depending on the experiment, the percentage of $CD1a^+$ cells co-expressing CD14 varied between 10 and 70%. The results presented in Table 1 below were obtained from experiments in which less than 15% of $CD1a^+$ cells co-expressed CD14; thus, both $CD1a^+$ cells and $CD1a^-CD14^+$ cells were characterized. $CD1a^+$ cells co-expressed CD1c, CD4, and CD40, but did not express CD1b. In contrast, $CD1a^-CD14^+$ cells did not express CD1C and only weakly expressed CD4 and CD40. Both $CD1a^+$ and $CD14^+$ cells were found to bear $Fc\gamma$ RII (CD32), $Fc\gamma$ RIII (CD16), and CR3 (CD11b), while only $CD1a^-CD14^+$ cells expressed $Fc\gamma$ RI (CD64) and CR1 (CD35). $CD1a^+$ and $CD1a^-CD14^+$ cells expressed both LFA1α (CD11a) and LFA1β (CD18), but $CD1a^+$ cells exhibited higher levels of ICAM1 (CD54) than $CD1a^+CD14^+$ cells. $CD1a^+$ cells expressed very high levels of HLA-DR (5-10 times more than $CD14^+$ cells). In contrast, $CD1a^-CD14^+$ cells expressed high levels of $HLA\text{-}DQ^+$.

TABLE 1

Phenotype of Dendritic Cells of the Invention

Antigen	Antibody	Reactivity with CD1a cells	Reactivity with CD14 cells	Source of Antibody (*)
CD1a	OKT6, IOT6a, T6, DMC1, L544	++	-	Ortho, Hy8, Imu, Cou,
CD1b	IOT6b	-	-	Imu
CD1c	IOT6c	+	-	Imu
CD14	Leu-M3	+/-	+++	BD
CD4	IOT4	++	+/-	Imu
CD40	Mab 89	++	+/-	EP
CD16	Leu11b	+	+	BD
CD32	2E1	+/-	+/-	Imu
CD64	197	-	+	Med
CD21	CR2	-	-	BD
CD11b	IOM1	++	++	Imu
CD35	IOT17	-	++	Imu
CD54	84H10	+++	+	Imu
CD11a	SPVL7	++	++	Hy2
CD18	BL5	++	++	Imu
HLA-DR	L 243	++++	++	BD
HLA-DQ	SPVL3	+++	+	Imu

* Antibodies were obtained from the following sources: Ort=Ortho Diagnostic Systems; Imu=Immunotech; Cou=Coulter (Hialeah, FL); BD=Becton-Dickinson; Med=Medarex Inc. (Lebanon, NH); Hy2=Hybridoma, Vol. 2, pg. 423 (1983); Hy8=Hybridoma, Vol. 8, pg. 199 (1989); EP=Eur. Pat. Appl. 89 403 491.7

Example 2

Stimulation of CD4 T cells

Dendritic cells of the invention were assayed for their capacity to induce resting allogenic $CD4^+$ T cells to proliferate. As shown in Fig. 3A, cells cultured in the presence of IL-3 alone induced marginal allogenic $CD4^+$ T cell proliferation. In contrast, cells cultured in the presence of GM-CSF alone, IL-3 plus TNFa, and GM-CSF plus TNFa induced a strong proliferation of allogenic $CD4^+$ T cells. Depending on culture conditions of the $CD34^+$ progenitor cells, the optimal proliferation of the $CD34^+$ T cells was observed for different values of the ratio (stimulator cells)/(allogenic $CD34^+$ T cells). When compared to control values ($CD34^+$ T cells without stimulator cells), a 50-fold enhancement of tritiated thymidine uptake by $CD4^+$ T cells was observed at a ratio of 1:3.8 (range 1:3 to 1:25), 1:12.5 (range 1:10 to 1:35), and 1:360 (range 1:100 to 1:400) for cells cultured in the presence of GM-CSF alone, IL-3 plus TNFa, and GM-CSF plus TNFa, respectively (ranges from 5 experiments). Fig. 3B shows that allogenic $CD4^+$ T cells derived either from cord blood or from adult peripheral blood were equally stimulated and that syngenic $CD4^+$ T cells were stimulated to a much lower extent (response 20-fold weaker than allogenic cells). When $CD1a^+$ cells were removed by immunomagnetic depletion (Fig.4), a strong loss of induction capacity was observed. A 50-fold enhancement of $CD4^+$T cell proliferation was observed for a ratio, (stimulator cells)/(allogenic $CD4^+$ T cells) of 1:200 (range of 3 experiments 1:200 to 1:400) and 1:8 (range of 3 experiments 1:8 to 1:40) before and after $CD1a^+$ cells were depleted, respectively.

Example 3

Generation of Cancer-specific CD8 Cytotoxic T Cells for Adoptive Immunotherapy

$CD34^+$ cells are isolated from the peripheral blood of a cancer patient and grown in the presence of GM-CSF and TNF-α, as described above. Peripheral blood CD8 cells are cryopreserved. Once dendritic cells are generated, CD8 T cells are thawed and mixed with the patient's cancer cells. After sensitization, CD8 T cells are expanded in the presence of IL-2, e.g. as described by Rosenberg, U.S. patent 4,690,915. Total cells are reinfused into the patient provided they display tumor specific cytotoxic activity. The tumor cells may be replaced by specific tumor antigens, e.g. van der Bruggen et al, Science, Vol. 254, pp. 1643 (1991).

Example 4

Generation of Primary and Secondary Antibody Responses in SCID-hu Mice

SCID-hu mice have allowed secondary responses to recall antigens but have not permitted the establishment of primary responses, e.g. Moller, The SCID-hu Mouse, vol. 124 (Munksgaard, Copenhagen, 1991); Duchosal et al, Nature, vol. 355, pp. 258 (1992); and Mosier et al, Curr. Top. Microbiol. Immunol., Vol. 152, pp. 195 (1989). It is believed that this is due to a lack of reconstitution of the dendritic cell pool. As antigen-pulsed dendritic cells can efficiently induce an antibody response in vivo, e.g. Sornasse et al, J. Exp. Med., vol. 175, pp. 15 (1992), SCID-hu mice are reconstituted with the dendritic cells generated in vitro from the CD34 cells of the donor of human cells or from another donor sharing a compatible MHC. Dendritic cells are pulsed with the appropriate antigen prior to injection. Once the mouse displays antibody to the antigen, B cells are isolated from blood and other reconstituted organs and immortalized, e.g. by culturing in the CD40 system in the presence of Epstein-Barr virus as taught by Banchereau et al, Science, Vol. 251, pp. 70 (1991).

Example 5

Treatment of AIDS by Adoptive Immunotherapy with Dendritic Cells Generated in Vitro

Dendritic cells are infected by HIV in vitro and HIV is found budding from the cell surface of the cells after 3-5 days in culture with HIV, e.g. Patterson, J. Gen. Virol., Vol. 68, pgs. 1177-1181 (1987); Macatonia et al, Immunology, vol. 71, pgs. 38-45 (1989); and Knight et al, Immunol. Lett., Vol. 19, pgs. 177-182 (1988). Evidence for in vivo infection of dendritic cells by HIV comes from the description of infection of Langerhans's cells of the skin and reduction in the amount of MHC class II molecules in cells of the skin,

e.g. Tschachler et al, J. Invest. Dermatol., Vol. 88, pgs. 233-237 (1987); and Belsito et al, N. Engl. J. Med., vol. 310, pgs. 1279-1282 (1984). Furthermore, 3-21% of blood dendritic cells from HIV individuals contain HIV, with a level of infection two orders of magnitude greater than that seen in other cell types, Macatonia et al, Immunology, Vol. 71, pgs. 38-45 (1990). The infection of dendritic cells by the virus blocks their ability to present antigen to T cells, and thus inhibits the recruitment/proliferation of the T cells which is necessary for a successful immune response.

CD34$^+$ cells of HIV patients are used to generate dendritic cells in accordance with the invention. The dendritic cells are then incubated with selected antigens and reinfused into the HIV patient.

Claims

1. A mixed lymphocyte reaction comprising the steps of:
providing a sample of responder cells;
providing a sample of inactivated stimulator cells such that the stimulator cells are allogenic with respect to the responder cells and such that the stimulator cells consist of dendritic cells produced by the process comprising the steps of (a) treating CD34$^+$ hematopoietic cells with tumor necrosis factor-α and a cytokine selected from the group consisting of granulocyte-macrophage colony stimulating factor and interleukin-3, and (b) isolating treated CD34$^+$ hematopoietic cells that express the CD1a antigen;
co-culturing the responder cells and the inactivated stimulator cells; and measuring a response of the responder cells.

2. The method of claim 1 wherein said CD34$^+$ cells are treated with tumor necrosis factor-α and granulocyte-macrophage colony stimulating factor.

3. The method of claim 2 wherein said response of said responder cells is proliferation.

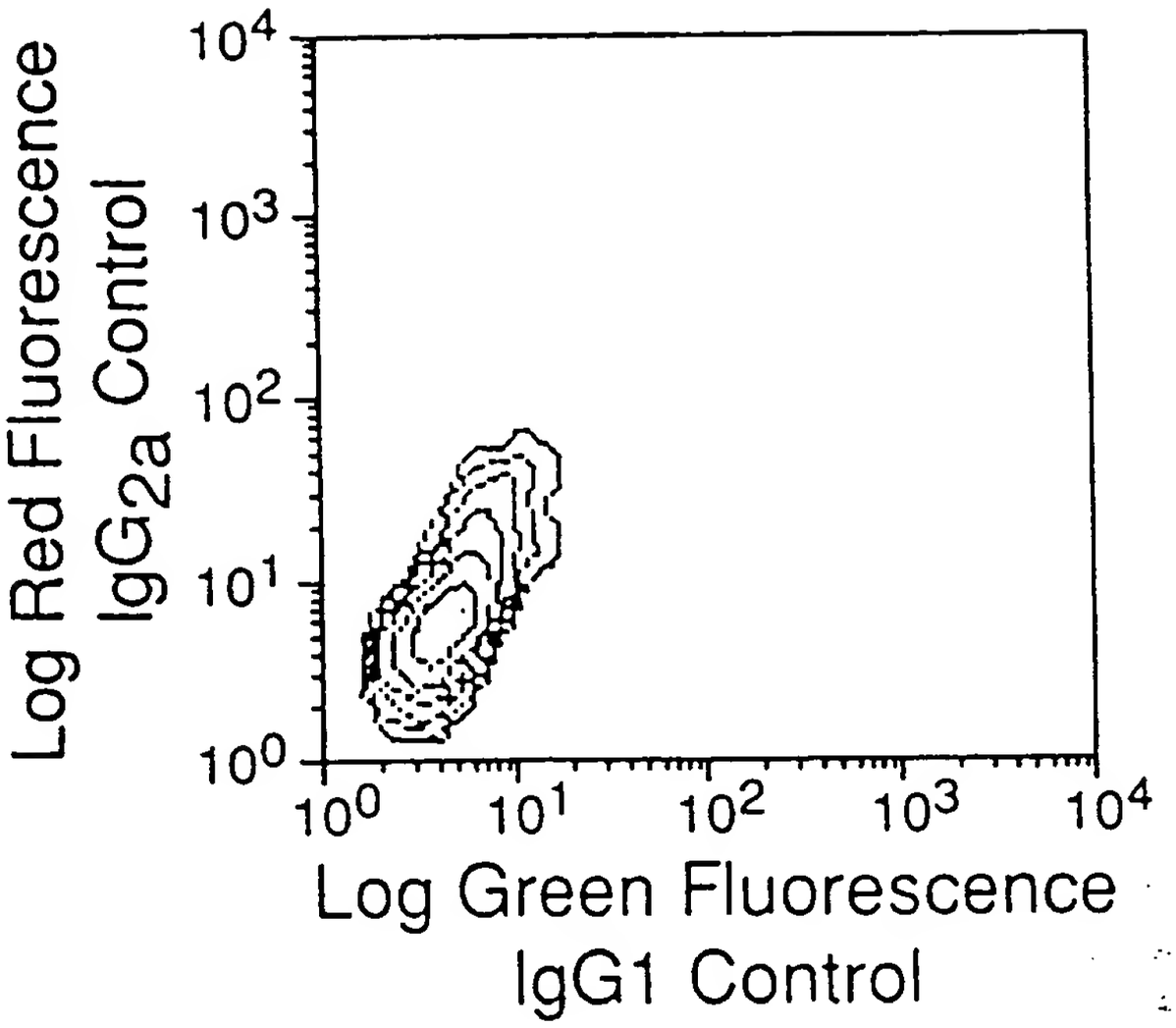
4. A method of preparing dendritic cells comprising the steps of:
a) treating CD34$^+$ hematopoietic cells with tumor necrosis factor-α and a cytokine selected from the group consisting of granulocyte macrophage colony stimulating factor and interleukin-3,
b) isolating treated CD34$^+$ hematopoietic cells that express the CD1a antigen.

5. The method of claim 4 wherein said CD34$^+$ cells are treated with tumor necrosis factor-α and granulocyte-macrophage colony stimulating factor.

6. A cellular composition comprising human dendritic cells produced by the process comprising the steps of:
treating CD34$^+$ hematopoietic cells with tumor necrosis factor-α and a cytokine selected from the group consisting of granulocyte-macrophage colony stimulating factor and interleukin-3, and
isolating the treated CD34$^+$ hematopoietic cells that express the CD1a antigen.

7. The cellular composition of claim 6 wherein said CD34$^+$ cells are treated with tumor necrosis factor-α and granulocyte-macrophage colony stimulating factor.

Fig. 1A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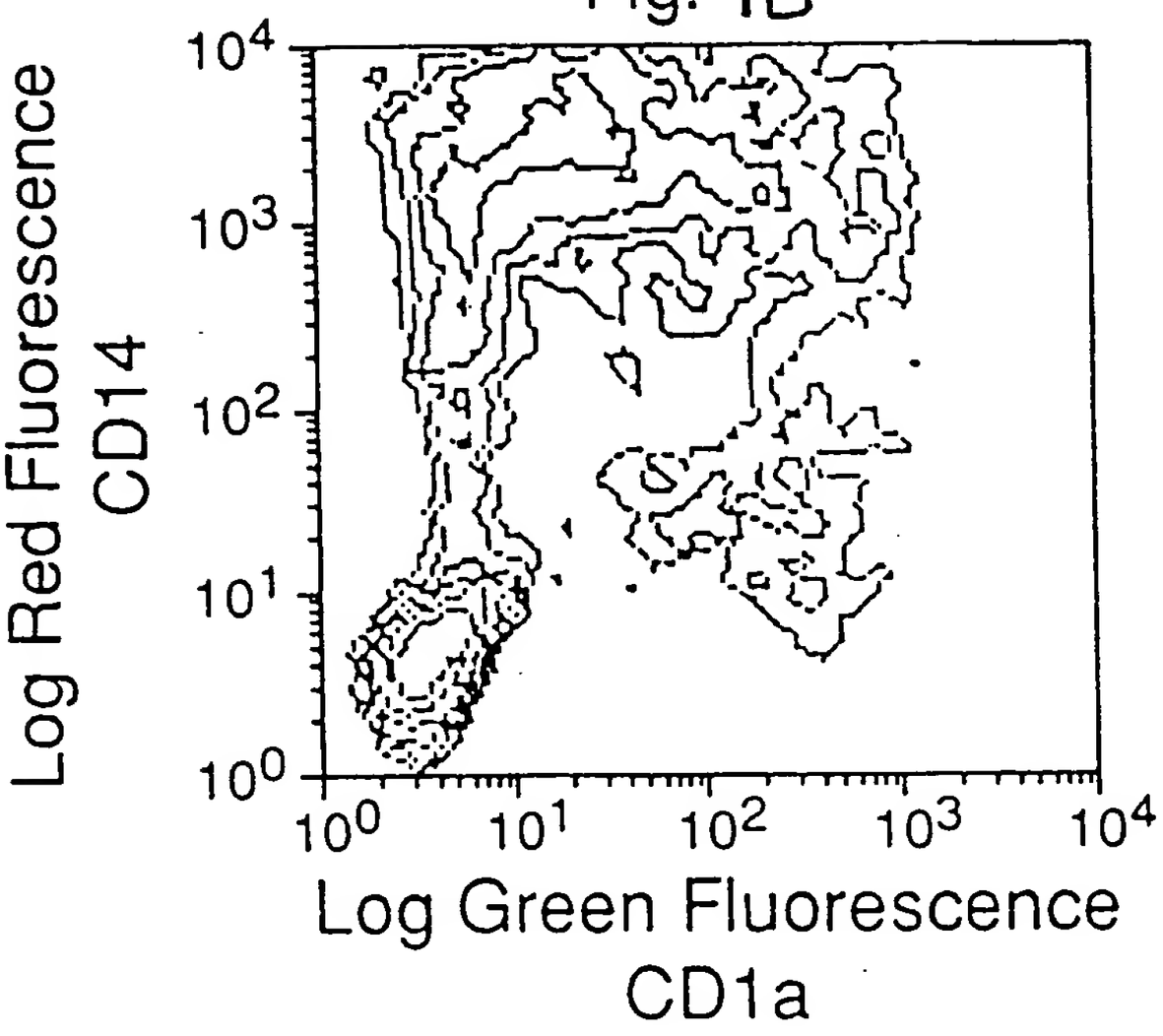
IgG2a Control
10^0
10^1
10^2
10^3
10^4
Log Green Fluorescence
IgG1 Control

Fig. 1B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CD14
10^0
10^1
10^2
10^3
10^4
Log Green Fluorescence
CD1a

Fig. 2A

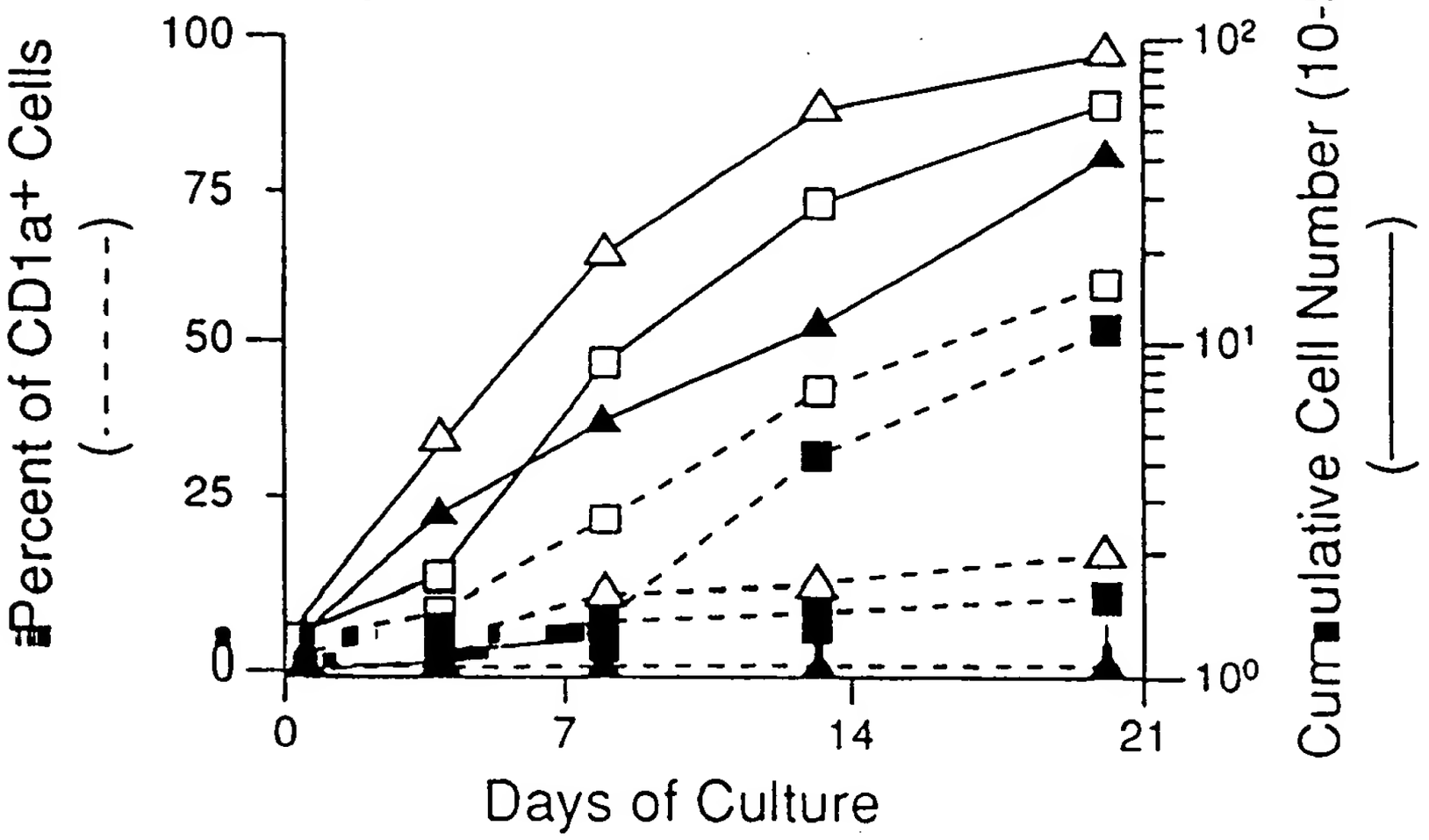

Percent of CD1a+ Cells
(------)
100
75
50
25
0
0
7
14
21
Days of Culture
10^2
10^1
10^0
Cumulative Cell Number (10-5)
(——)

Fig. 2B

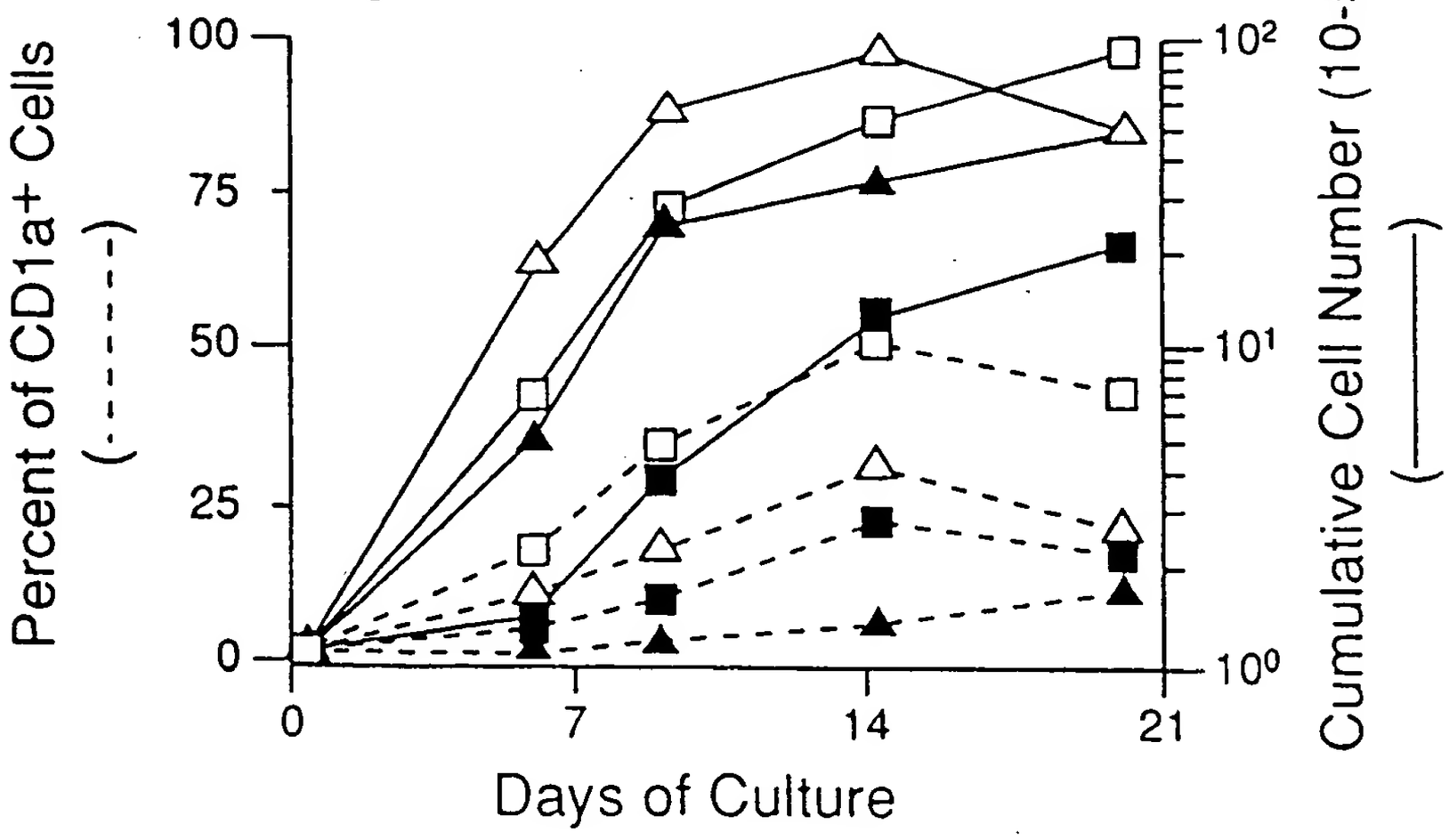

Percent of CD1a+ Cells
(------)
100
75
50
25
0
0
7
14
21
Days of Culture
10^2
10^1
10^0
Cumulative Cell Number (10-5)
(——)

Fig. 3A

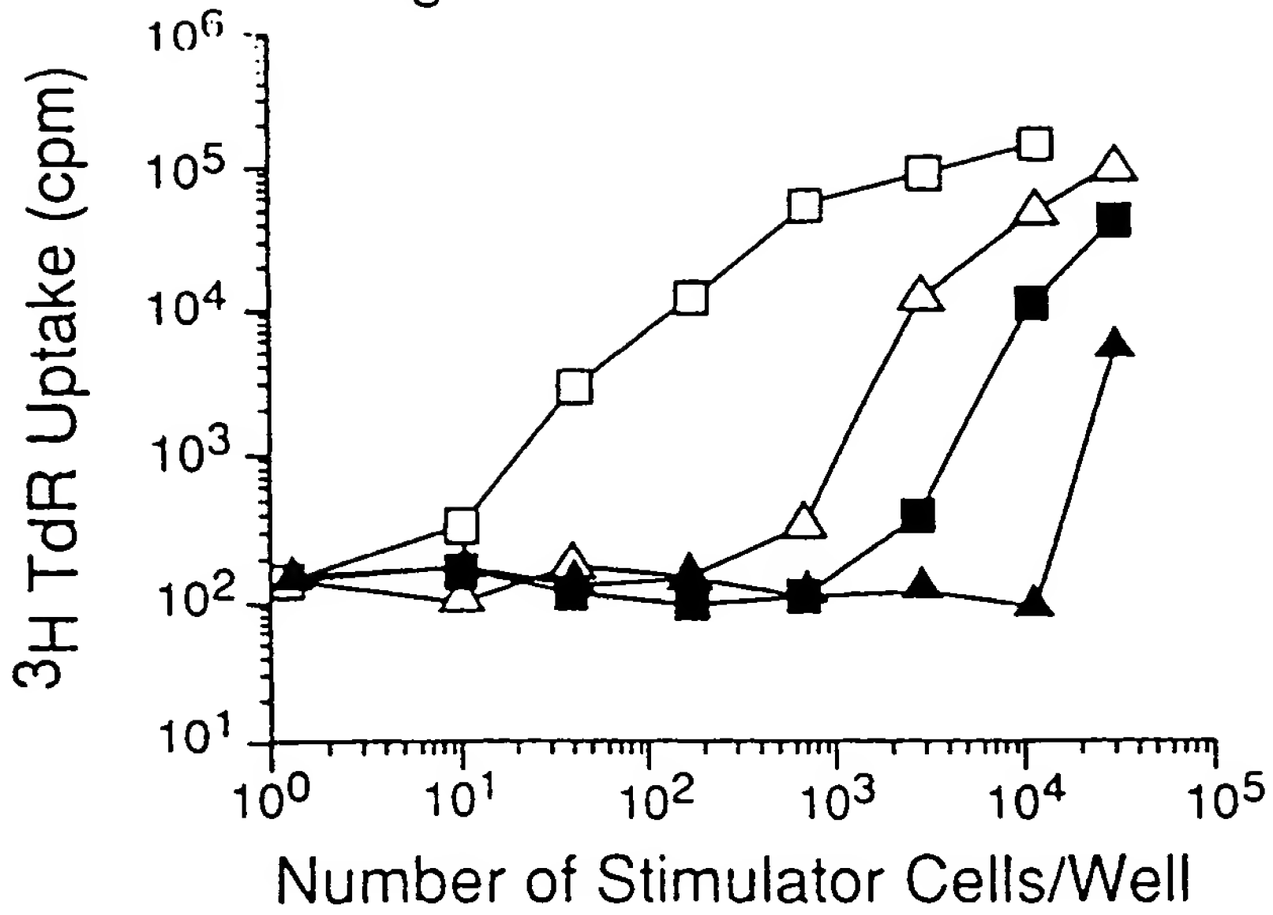

^{3}H TdR Uptake (cpm)
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^1
10^2
10^3
10^4
10^5
Number of Stimulator Cells/Well

Fig. 3B

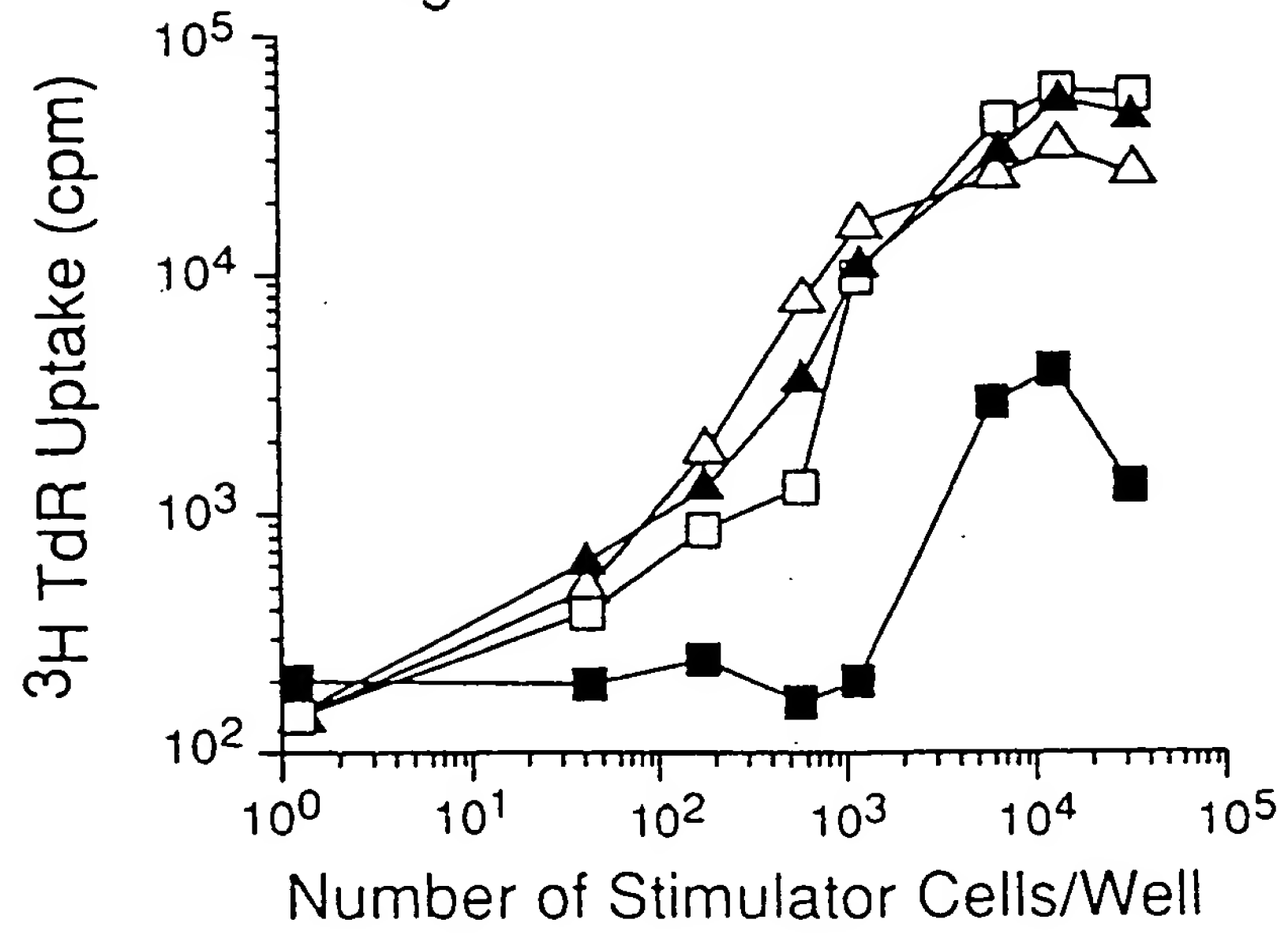
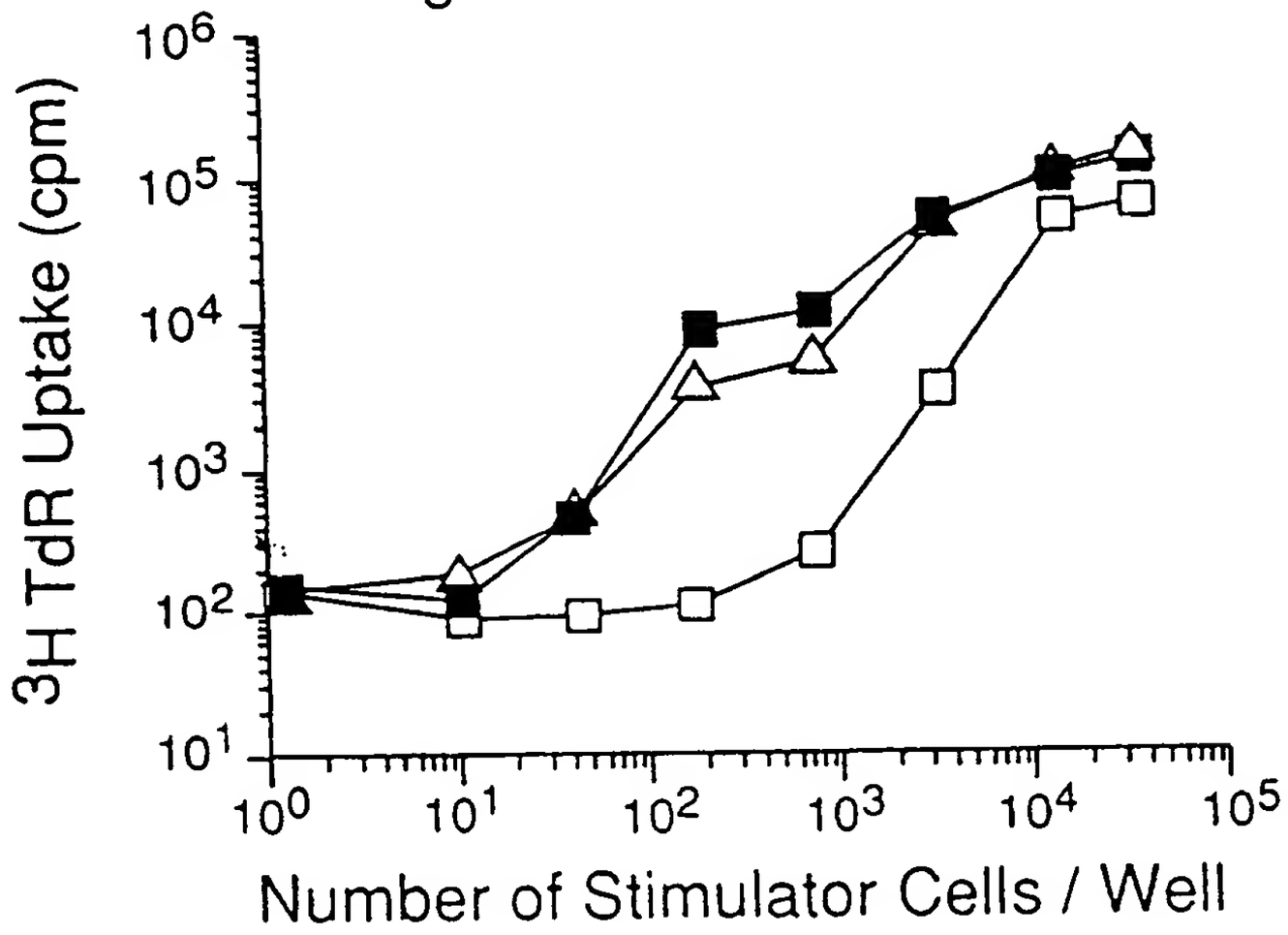

^{3}H TdR Uptake (cpm)
10^5
10^4
10^3
10^2
10^0
10^1
10^2
10^3
10^4
10^5
Number of Stimulator Cells/Well

Fig. 4


^{3}H TdR Uptake (cpm)
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^1
10^2
10^3
10^4
10^5
Number of Stimulator Cells / Well

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 40 0879

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	BLOOD vol. 75, no. 12, 1990, NEW YORK pages 2292 - 2298 CAUX C;SAELAND S;FAVRE C;DUVERT V;MANNONI P;BANCHEREAU J 'TUMOR NECROSIS FACTOR-ALPHA STRONGLY POTENTIATES INTERLEUKIN-3 AND GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR-INDUCED PROLIFERATION OF HUMAN CD34-POSITIVE HEMATOPOIETIC PROGENITOR CELLS' * the whole document *	1,4,6	C12N5/08
A	---	2,5,7	
Y	JOURNAL OF INVESTIGATIVE DERMATOLOGY vol. 97, no. 3, 1991, BALTIMORE US pages 524 - 528 C.MOULON ET AL. 'A potential role for Cd1a molecules...' * the whole document *	1,4,6	
A	--- JOURNAL OF INVESTIGATIVE DERMATOLOGY vol. 94, no. 2, 1990, BALTIMORE US pages 166 - 173 TEUNISSEN MB; WORMMEESTER J; KRIEG SR; PETERS PJ; VOGELS IM; KAPSENBERG ML; BOS JD 'Human epidermal Langerhans cells undergo profound morphologic and phenotypical changes during in vitro culture.' * the whole document *	1,4,6	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C12N C07K
A	--- WO-A-9 106 630 (HAHNEMANN UNIVERSITY) * the whole document *	1-7	
A	--- WO-A-9 102 531 (PETER MACCALLUM CANCER INSTITUTE) * claim 15 *	1,2,4-7	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	17 NOVEMBER 1992	GURDJIAN D.

CATEGORY OF CITED DOCUMENTS

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 455 482 (BECTON DICKINSON & CO.) * the whole document * -----	1-7	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
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